A. APPLICATION FOR PERMIT FOR SCIENTIFIC PURPOSES UNDER THE ENDANGERED SPECIES ACT OF 1973 (E.G. FOR FIELD SURVEYS, GENETICS RESEARCH ETC.)

B. SPECIES

Puget Sound Chinook salmon (*Oncorhynchus tshawytscha*)

C. June 15, 2015

D. 1. Tina Wyllie-Echeverria, Ph. D. Fisheries Oceanographer, Principal Investigator and owner
Wyllie-Echeverria Associates,
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E. Information on Personnel, Cooperators and Sponsors

1. Dr. Wyllie-Echeverria will act as Principal Investigator and Field Supervisor for these projects. Her resume is attached to this application.

2. Field Personnel:
Sandy Wyllie-Echeverria
Rebecca Wyllie-Echeverria
Tessa Wyllie-Echeverria
Victoria Wyllie-Echeverria
Russel Barsh
David Loyd

3. a. Salmon Recovery Funding Board is the grantor and the proposal is in the final round for consideration (January 2007) and scheduled to begin in February 2007. Mike Ramsey, SRFB 1111 Washington Street SE
P.O. Box 40917
Olympia, Washington 98504-0917, (360) 902-2969, Email: miker@iac.wa.gov

Samish Indian Nation is the grantee with Wyllie-Echeverria Associates in charge of the field sampling. Christine Woodard is the project manager for the Samish. Russel Barsh is the project manager for Center for the Ecology of the Salish Sea

4. A contract will exist between the Samish Indian Tribe and/or Center for the Ecology of the Salish Sea and Wyllie-Echeverria Associates to conduct all fieldwork. Since Dr. Tina Wyllie-Echeverria is the principal on this permit, no additional paperwork should be needed for this section.

5. Wyllie-Echeverria Associates will archive any tissue or dead specimens. Collaboration with the Genetics laboratory at NMFS, Montlake, Seattle WA will provide population information on the origin of the juvenile salmonids sampled from the San Juan Archipelago and archive the genetic tissue samples. A small amount of fin membrane will be removed from the anal or pelvic fin of the fish and sent to the laboratory for analysis. This is an accepted procedure on teleosts, which allows non-lethal sampling.

6. No collection or long-term holding of any living species is planned.

F. PROJECT DESCRIPTION, PURPOSE, SIGNIFICANCE

1. The principal project under SRFB funding is a one-year study whose objective is to clarify which salmonid species (and to a more limited extent, which Chinook stocks) utilize intertidal beaches or subtidal eelgrass meadows at representative sites on San Juan, Orcas, Shaw, Lopez, Jones and Waldron Islands, which are relatively exposed to the Fraser, Nooksack, and Samish watersheds. Direct evidence of species and stock specific utilization, as opposed to mapping of habitats (eelgrass) and prey availability (forage fish spawning) will improve our ability to set priorities for the protection of critical salmonid habitat in WRIA2, as prescribed by the WRIA2 salmon recovery strategy.

Although its terrestrial extent is small (177 square miles), San Juan County offers migrating salmonids approximately 408 linear miles of shoreline for foraging (Nearshore Habitat Program 2001). A large proportion of the shoreline is exposed bedrock, and the numerous rocky reefs of San Juan County have attracted recent conservation interest as habitat for rockfish and hexagrammids (Sato & Wyllie-Echeverria 2004). Sandy beaches can also be found on the larger islands, where they may support surf smelt, sand lance, and herring spawning (Miller et al. 1980; Cederholm et al. 2000). The habitats range from small pocket estuaries with less than an acre of intertidal substrate, typical of bedrock-dominated shorelines, to broad continuous exposed and relatively undisturbed ribbons of sand and gravel more than a mile in length. Numerous shallow bays and the three fiords of Orcas Island have eelgrass (*Zostera marina*) meadows that support diverse communities of fish and crustaceans (Phillips 1984; Simenstad 1994). San Juan County represents a large and geomorphologically diverse habitat matrix, directly in the path of salmonid migrations.

3. Juvenile salmonids are present in Puget Sound marine waters throughout the year (Rice et al. 2004). Juvenile chum, pink and Chinook were amongst the ten most common species found in beach seine collections made by Miller et al. (1977) around the San Juan Islands, and Miller reports that juvenile chum and Chinook continue to appear in beach seine collections made by his students at Friday Harbor Marine Laboratories. However, while students at FHML have beach seined for more than 20 years, the data are of limited value because the methods, sampling locations,

and times of year have varied from year to year (Moulton 2004). It is unknown (for example) whether stocks vary in their choice of habitats in the San Juan Islands, and whether they vary in residence time. The absence of a consistent methodology or design also means that existing data may underestimate the number and diversity of stocks that annually utilize San Juan Islands nearshore habitats.

4. The Salmon Recovery Funding Board funds several projects that will assess nearshore habitat use by juvenile salmonids in northern Puget Sound. Our proposal will be coordinated with the proposals for Island County. All SRFB proposals will work together to use common methodology and analysis. This will be the first attempt to coordinate multi-WRIA projects into a comprehensive mission. The project managers of all proposals will coordinate methodologist and data analysis even if the proposed coordinating grant is not funded. The importance of comparable information for the North Sound is essential, as the habitats are similar and techniques applicable to all these nearshore habitats.

5. The WRIA2 Lead Entity Citizens Committee is in the final stages of preparing its salmon recovery strategy. The draft WRIA2 *Handbook of Salmon Recovery in San Juan County* (June 2004) states at page 86: "The most critical data gap in our knowledge of the role played by WRIA 2 in salmon ecology is knowing the location, extent and landscape patterns of habitats essential for the early marine life history of salmon and for the prey species upon which out-migrating juveniles and in-migrating adults feed."

G. Project Methodology

1. Duration: January 2007- December 2009

2. A. Intertidal fish presence will be determined by beach seining after Beamer et al. (2003). An 80-foot seine is deployed by hand in 1-4 feet of water from a floating tub or dinghy, and then pulled ashore by a crew of four. All fish are identified and counted before returning them to the water; the first 20 individuals of each species are also measured to determine their average length. Salmon are handled gently with surgical gloves to avoid scale loss and minimize stress. Chinook are weighed, examined visually for external marks, and checked for coded wire tags with an electronic "wand." Two successive seine sets are carried out at each site as replicates, typically at the speed of one set every 15-20 minutes, moving up the current to minimize catching the same fish. Processing the catch from each set may take an hour or longer depending on the number of fish landed, which in our experience thus far has usually been under 100, but occasionally over 1,000. Salmonids will be processed first to expedite their return to the Sound. All fish will be processed on site and returned to the area of capture.

Genetic samples will be collected from anal fin tissue of sedated fish. A 2mm diameter sample of tissue will be collected and stored in ethanol. This method allows investigation of DNA and works well for releasing fish alive. A total sample size of 50-

100 fish will be collected in 2007 and again in 2008 to ensure a representative sample for a population.

A 10' diameter toss net will be used to sample fish from areas over deep water but inaccessible to trawls and seines. The toss net is designed to sample fish that will be kept alive. We will toss three replicate samples around docks and rocky areas. Sampling will be done from a boat or dock and the fish will be returned to the area from which they were caught. The fish will be processed in the same manner as seine-caught fish.

A surface tow net deployed by two boats will be used to sample mid- channel populations. Casimire Rice, NMFS used this technique in 2003-2004 to sample juvenile salmon population in central Puget Sound. The same gear and techniques will be used to sample around the San Juan Islands in 2005. We will contract with a 54' former seine boat for the deployment of the net at each site. We will use the "Coral Sea" to sample the channel habitats one day each month during April, May and June. We are targeting the offshore areas adjacent to the nearshore sampling sites to determine if juvenile salmonids are distributed throughout the area or localized nearshore. All fish will be identified at the completion of a tow and returned to the area from which they were caught. The fish will be processed in the same manner as seine-caught fish.

B. . The planned schedule is to sample monthly between April through September. Six locations will be sampled: Waldron Island (Cowlitz Bay and Seversen's Beach), West Beach on Orcas Island, two sites on Shaw. Each location will be sampled with four seines (two with the 80' and two with the 120'). Each site will also be sampled with a toss-net around piers and marinas and rocky reef habitat. Replicate toss-net samples will be taken at each location. During April, May and June mid-channel tows will be made at the three locations. Two tows at each location. We plan to perform the sampling over six days within a two week period each month.

Waldron Island N48o42' W123o 3'
West Sound, Orcas Island N48o 37' W123o 0'
San Juan Channel (Shaw and San Juan Islands) N48o 32' W123o 0'
West Beach, Orcas Island N48o 37.4' W123o 57'
Hick Bay, Shaw N48o33.0 W W123o58.0'
Wasp Passage, Shaw N48o35' W123o 0.0'

C. Coded wire tag and elasomer external tags will be used to mark Chinook for tracking behavior within nearshore environments. This would answer the question as to whether the fish are moving through the cove in waves or the same fish staying in one localized area for a period of time. Northwest Marine Technologies is assisting in the planning of this section and will train and assist in the tagging process. For fish tagged with a coded wire tag the tag will be placed in the opercular bone, on either side of the dorsal fin and either side of the caudal fin in order to distinctively mark 5 sets of juvenile fish. 100-200 fish will be marked and released is 5 separate coves in

San Juan County for a total number of tagged fish numbering between 600-1200. Fish will be recaptured and released once the presence and position on the fish of the tag has been determined. Fish will originate from the Glenwood Springs Hatchery on Orcas Island which is not a hatchery listed on the ESA hatchery list. We will also place a color coded elasomer tag on the caudal fin membrane for easy identification of CWT fish. Five colors will be used, one for each cove where the 100-200 fish will be released.

D-E. Finquall (MS222) will be used to sedate the subsample of Chinook while they are being measured and weighted or tagged. A rapid temporary immobilization technique will be followed to allow for rapid immobilization and rapid recovery. The dose would be 100 ppm for a response time of 2-5 minutes, the fish will be narcotized for approximately 15 minutes during which we will measure and weigh them, return them to a bucket with fresh seawater until they have recovered and release them into the Sound at the location from which they were sampled. We use MS222 for all our studies that require careful handling of specimens and to reduce damage and shock to the fish. It is commonly used to transport fish, during tagging studies and operations, during gastric lavage, and measurements.

F. Genetic samples will be collected form anal fin tissue of sedated fish. A 2 mm diameter sample of tissue will be collected and stored in ethanol. This method allows investigation of DNA and works will for releasing fish alive. A total sample of 50-200 fish will be collected in 2007-2008 to ensure a representative sample for a population. Fin clips will be collected from a subsample (N=10) of Chinook, Coho, and chum salmon per site per year in order to determine their stream or watershed of origin. No long-term adverse affects are expected from taking a fin clip as we expect regeneration of fins to occur. Caudal fin regeneration in zebra fish has been documented at 65% in 7 days in clinical trials (Zudrow and Tanguay 2003).

3. The main technique used to sample fish in the nearshore will be the beach seine. Several other techniques may be employed if the fish do not come close enough to shore for the beach seine. These techniques are described in section 2A and include tow-net, toss net, or fyke net.

4. Landing fish in any type of net crowds them together so that they can rub against one another and lose scales. As we bring in the nets we either keep the fish loosely gathered in the net with the net remaining partially submerged (beach seine, fyke net) or immediately transfer the fish into a large holding tank once the net is brought aboard the ship (tow net, toss net). The goal is to reduce any injury due to crowding. Once the fish are in the holding tanks two initial sorts occur. Predatory fish such as the staghorn sculpin are removed into a separate tank and all salmon are sorted into their own holding tank. Chinook are further separated for obtaining weights and length data and possible genetic tissue and are anesthetized as described in section G-2.D-E. All fish are retuned to the site where they were collected by gently submerging the buckets that are holding up to 100 fish into the water and letting the fish swim out.

Their behavior is observed and any mortalities are collected and used for further genetic or prey analysis.

H. Take

1. Puget Sound Chinook salmon, status and trends:
The research area addressed in this application may contain Puget Sound Chinook from a wide range of streams and hatcheries. The closest populations would come from the Nooksak, Samish and Skagit Rivers as well as rivers and streams in British Columbia. Hatcheries throughout this area could also contribute to the populations in San Juan County. During the 2004-06 sampling seasons we did not capture any coded-wire tagged or adipose fin clipped hatchery fish. However, fish from the Kendall Creek Hatchery and Lummi Hatchery could be in the study area. Since we are sampling wild, or unmarked, Chinook we are providing information on wild stocks. With the genetic analysis scheduled for 2006-2007 we will be able to directly determine which watersheds are contributing to the San Juan County (WRIA 2) juvenile Chinook populations. Studies in the Skagit River delta have shown a poor recruitment of juvenile Chinook to nearshore pocket estuaries following the 2004 drought year (Beamer et al. 2006). Population distribution of wild Chinook in pocket estuaries followed the trend of fewer wild fish leaving the watershed in 004 than in 2005. Inter-annual variation in populations due to climate and/or fresh water habitat conditions must be integrated into the trends model in order to track the cause and affect of population fluctuations once the fish are in the marine environment.

2. We expect about 1% mortality from handling, based on sampling using established methods in Puget Sound. The sample will be held in a large container of fresh seawater and Chinook salmon sorted out of the general catch as the first order of working up the sample.

Take estimates are based on sampling during 2004 and discussions with other biologists sampling in the Puget Sound. We should be able to stay within the 1% mortality window we are estimating. Sampling protocol training sessions will be held to standardize the procedures among several groups and to gain knowledge in the care and handling of juvenile Chinook. We suspect most of the salmonids we see in San Juan County originate in the Fraser River system but will know more after this year's genetic analysis.

3. We expect to encounter naturally-produced juvenile Puget Sound Chinook salmon, and will minimize lethal sampling by collecting only fin clips and unintentional mortalities; we are proposing a total annual ceiling of 15 Chinook from San Juan County. We will handle the juvenile salmon with extreme care. At this time, we do not know the origin of the juvenile salmon that we will encounter. This study will add to that knowledge through genetic identification. We can identify three "take" categories: Capture/release with a 1% potential unintentional mortality rate, Capture/handle/release release with a 1% potential unintentional mortality rate, and capture/fin clip/release release with <1% unintentional mortality rate.

4. During 2004-2006 we have not caught any species listed by USFWS and do not anticipate doing so in 2007-2008. Our sampling techniques will remain the same and we have not captured any Bull Trout or have a record of anyone catching Bull Trout in our geographic area.

Summary Table:

ESU/Species	**Life Stage**	**Origin**	**Take Activity Category**	**Number of Fish Requested**	**Requested unintentional Mortality**	**Location**	**Date(s)**
Puget Sound Chinook Salmon	Juvenile	Naturally produced (Wild)	Capture/Handle / Release	1500	15	San Juan Islands	April - Sept
Puget Sound Chinook Salmon	Juvenile	Naturally produced (Wild)	Capture/Fin Clip/Release	50	N/A	San Juan Islands	April - Sept
Puget Sound Chinook Salmon	Juvenile	artificially-propagated (hatchery) with intact adipose fins	Capture/Handle / Release	1500	15	San Juan Islands	April - Sept
Puget Sound Chinook Salmon	Juvenile	artificially-propagated (hatchery) with intact adipose fins	Capture/Fin Clip/Release	50	N/A	San Juan Islands	April - Sept
Puget Sound Chinook Salmon	Juvenile	artificially-propagated (hatchery) with clipped adipose fins	Capture/Handle / Release	1500	15	San Juan Islands	April - Sept
Puget Sound Chinook Salmon	Juvenile	artificially-propagated (hatchery) with clipped adipose fins	Capture/Fin Clip/Release	50	N/A	San Juan Islands	April - Sept
Puget Sound Chinook Salmon	Juvenile	artificially-propagated (hatchery)-tagged	Tag/Release	1200	N/A	San Juan Islands	April - Sept

I. Transportation

a. Capture and release is on site. Tagged fish from the Glenwood Spring Hatchery will be transported to selected coves under the mandate of Washington State Department of Fish and Wildlife. A stocking permit and transport authorization is being obtained from the State. Transport will be via holding tanks on a vessel from the tagging site (Orcas Island) to the sites of release (Waldron, Lopez, Shaw, Orcas Islands).

J. Cooperative breeding Program

We are willing to cooperate with a breeding program.

K. Previous Activity with listed species

1. Tina Wyllie-Echeverria has been a primary investigator working with federally listed species under permit number 1521 for 2005-2006.

ESU/Species and population group if specified in your permit	**Life Stage**	**Origin**	**Take Activity**	**Actual Number of Listed Fish Taken**	**Actual Unintentional Mortality**	**Research Location**	**Research Period**
PS Chinook Salmon	Juvenile	Naturally Produced	Capture, Handle, Release	20	0/20	Nearshore habitats of Waldron and Orcas Islands, Washington	January-December 2005
PS Chinook Salmon	Juvenile	Naturally Produced	Capture, Handle, Fin Clip, Release	20	**0/20**	Nearshore habitats of Waldron and Orcas Islands, Washington	January-December 2005

1. Measures taken to minimize effects on listed fish and the effectiveness of these measures.

Fish were anesthetized (MS222) before fin tissue was collected, recovery in fresh sea water and returned to the sea after mobility was regained (total handling time was 2 minutes per fish)

2. How listed fish were injured or killed and how were they disposed of.

No mortalities during 2005 sampling efforts

3. Any problems that were encountered during the activities.

All sampling was performed successfully within the plan of the research project

L. Certification

"I hereby certify that the forgoing information is complete, true and correct to the best of my knowledge and belief. I understand this information is submitted for the purpose of obtaining a permit under the Endangered Species Act of 1973 (ESA) and regulations promulgated thereunder, and that any false statement may subject me to the criminal penalties of 18 U. S.C. 1001, or to penalties under the ESA.:

_____ _____

Tina Wyllie-Echeverria, Principal Investigator Date

References

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